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Science Advisory Report 2015/016

EVALUATING TRANSFERS OF HARVESTED SHELLFISH PRODUCTS, FROM THE WEST TO THE EAST COAST OF VANCOUVER ISLAND, AS A POTENTIAL VECTOR FOR EUROPEAN GREEN CRABS (*CARCINUS MAENAS*) AND OTHER NON-INDIGENOUS INVERTEBRATE SPECIES

European Green Crab (Carcinus maenas). Photo: S. Robinson, DFO.

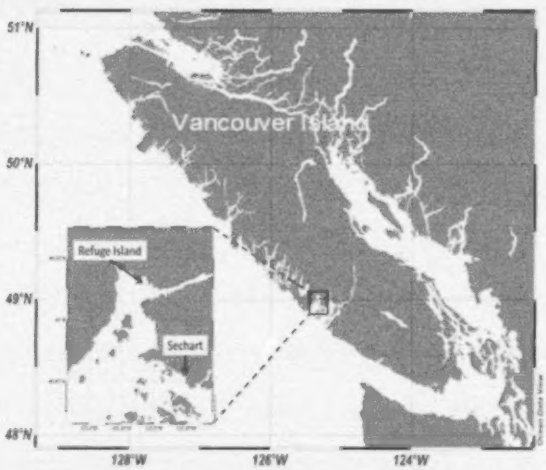

Figure 1. Experimental field sites at Refuge Island and Sechart, located in the northeast portion of Barkley Sound on the west coast of Vancouver Island, British Columbia.

Context:

Wild and cultured Manila Clams (Venerupis philippinarum) and Pacific Oysters (Crassostrea gigas) harvested on the west coast of Vancouver Island must be sent to processing plants on the east coast of Vancouver Island and lower mainland (in the Strait of Georgia), as no commercial shellfish processing facilities exist on the west coast of the island. Historically, processors were allowed to "wet store" large quantities of harvested west-coast product in the intertidal zone next to their plants or on aquaculture tenures (including those on the east coast of Vancouver Island), processing product as time and markets allowed. There were concerns, however, that this practice could lead to the spread of non-indigenous species (NIS), including European Green Crabs (Carcinus maenas), which are currently found along the west coast of Vancouver Island but not in the Strait of Georgia. As a result, in 2010, Fisheries and Oceans Canada (DFO) Aquaculture Management Division (AMD) added clauses to the shellfish culture licences that restricted wet storage and required visual inspection and rinsing of all shellfish product harvested on the west coast of Vancouver Island in an attempt to limit possible movement of C. maenas into the Strait of Georgia. Advice was also requested from the Science Sector on determining whether or not these shellfish transfers were a possible vector for movement of C. maenas and other NIS from the west to the east coast of Vancouver Island and, if so, how effective rinsing and visual observation are at mitigating the risk of such potential movement.

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This Science Advisory Report is from the December 3-4, 2013 meeting on Evaluating transfers of harvested shellfish products, from the west to the east coast of Vancouver Island, as a potential vector for European green crabs (Carcinus maenas) and other non-native invertebrate species. Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

SUMMARY

- Fisheries and Oceans Canada (DFO) Aquaculture Management Division (AMD) requested science advice on the shellfish aquaculture licence condition requiring that all shellfish cultured on the west coast of Vancouver Island be rinsed and visually inspected for European Green Crabs (*Carcinus maenas*) before transport to processing plants on the east coast of Vancouver Island and the lower mainland. Specifically, AMD wanted to know if shellfish can be a vector for Green Crab movements and, if so, what mitigation measures could be used to reduce the risk of their transport (the ultimate aim being to prevent *C. maenas* from being transported from the west coast of Vancouver Island, where populations currently exist, to the Strait of Georgia where the species has not yet been found).
- DFO Science conducted a field study to assess three species of shellfish (Pacific Oysters, *Crassostrea gigas*; Manila Clams, *Venerupis philippinarum*; and California Mussels, *Mytilus californianus*) as potential vectors for Green Crabs and other NIS. In addition, oysters and clams collected directly from growers and processors were examined for *C. maenas* and other NIS. A literature review was conducted to assess possible mitigation measures for NIS transport on shellfish.
- The transfer of shellfish (clams, oysters, and mussels) from NIS-infested areas was confirmed as a vector for both mobile (*e.g.* Green Crabs) and sessile (*e.g.* tunicates/ bryozoans) NIS.
- The potential propagule pressure due to the shellfish movement vector may be sufficient to overcome invasion barriers.
- Based on a literature review, no mitigation measures to remove or destroy NIS on cultured shellfish are 100% effective, suggesting any transfer of shellfish poses some level of invasion risk.
- The only mechanism to ensure NIS are not inadvertently moved from infested to non-infested waters via this vector is to restrict all movement of shellfish (*i.e.* strict quarantine).
- The results of the experimental and grower/processor studies and the literature review suggest that the present conditions of licence do not eliminate NIS propagule pressure. There are gaps in our knowledge surrounding various stages of the transport process (*e.g.* efficacy of rinsing in removing small individuals, survivability of individuals during transport, escape of live NIS from processing facilities, survivability in the receiving ecosystem). Due to these gaps it is probable that the intended reduction in propagule pressure is not realized.
- A conceptual framework was developed to identify control points where management intervention, such as application of licence conditions, could lower propagule pressure and hence invasion risk. A full assessment of the relative effectiveness of each control point in the framework was beyond the scope of this project.

INTRODUCTION

European Green Crabs (*Carcinus maenas*) were first detected on the west coast of North America in 1989 and had reached western Canadian waters by 1999 (Cohen and Carlton 1995; Gillespie et al. 2007). Directed surveys have delineated the distribution of this invasive species along the west coast of Vancouver Island, in addition to Sooke Harbour and the central coast of BC (Gillespie et al. 2007), with no confirmed reports from the Strait of Georgia (east coast of Vancouver Island). In 2010, DFO AMD put several stipulations on the transfer of shellfish aquaculture products from the west to the east coast of Vancouver Island, via conditions of licence, in an effort to limit the potential movement of Green Crabs from the west to the east coast of Vancouver Island. These conditions are highly relevant since a processing facility for shellfish does not exist on the west coast of Vancouver Island, and therefore all harvested shellfish are transferred to processors in the southern Strait of Georgia. These licence conditions were put in place as a precautionary measure and DFO Science did not provide specific advice to inform these conditions at that time. In 2011, DFO AMD formally requested advice from DFO Science on these licence conditions. Specifically, they wanted to know the potential for transporting NIS, especially invasive European Green Crabs, on cultured shellfish products, and if mitigation measures existed that could be used to reduce the risk of such transfers.

As a result, DFO Science conducted a research project through its Program for Aquaculture Regulatory Research (PARR) from July 2011 to October 2013 in an effort to characterize the movement of NIS on shellfish product transferred from the west to the east coast of Vancouver Island. The request for scientific advice from AMD came in the form of five separate objectives, which were as follows:

i. Identification and review of the potential processes by which the transfer of a range of cultured shellfish products provide a mechanism by which non-target aquatic invertebrate invasive species may be relocated to new ecosystems using present aquaculture processes.
ii. Description of the attributes of European Green Crab that could influence their ability to establish populations in a receiving ecosystem.
iii. Description of the range of transfer potential of European Green Crab as measured through experimental research and as extrapolated to current and historical commercial shellfish transfers. Provision of considerations around areas of uncertainty and assumptions introduced during the experimental research and extrapolation process.
iv. Evaluation of whether the information, data, and analysis presented for European Green Crab can be used to provide advice on the potential for current bivalve harvest practices to be a vector for other non-commercially harvested aquatic invertebrate invasive species.
v. Advice on potential mitigation measures, including their efficacy, which may be utilized to reduce the potential for transfer of non-target aquatic invertebrate invasive species.

This document forms the basis for scientific advice related to these specific objectives. It includes the results of experimental studies, observations, and analysis of historical data to assess the potential for the transfer of NIS, especially European Green Crabs, on cultured shellfish. Though the experimental work focuses on the movement of cultured bivalves, the stages of the invasion process, study results, and mitigation measures discussed are relevant to the transfer of all shellfish whether it is cultured, used for monitoring programs, or part of a wild commercial fishery. Further, it should be noted that shellfish transfers of both wild and commercially cultured species are not the only means of spreading NIS to new areas, as there are many other vectors that can transport NIS. These include recreational boating (Darbyson et al. 2009; Davidson et al. 2010; Rothlisberger et al. 2010; Clarke Murray et al. 2011;

Lacoursière-Roussel et al. 2012), commercial shipping (both ballast water [Carlton 1987; Cariton and Geller 1993; Ruiz et al. 2011; Wasson et al. 2001; Briski et al. 2012] and hull fouling [Ruiz et al. 2000; Davidson et al. 2010; Sylvester et al. 2011]), and live transport of marine species for bait, aquaria, and seafood (Chapman et al. 2003; Weigle et al. 2005; Keller and Lodge 2007). In addition to shellfish transfers, there are also other practices employed by the shellfish culture industry that are potential NIS vectors. These include de-fouling procedures (*e.g.* power washing) (Bock et al. 2011; Morris and Carman 2012) and spat collection (Darbyson et al. 2009).

ASSESSMENT

Methods

Two separate studies were conducted to determine if NIS entrainment occurs on cultured shellfish exported from the west coast of Vancouver Island. The first was an experimental field study conducted at two sites, Refuge Island and Sechart, in Barkley Sound (on the west coast of Vancouver Island) (Fig. 1) that ran from July 2011 to December 2012. Three species of shellfish (Pacific Oysters, *Crassostrea gigas*; Manila Clams, *Venerupis philippinarum*; and California Mussels, *Mytilus californianus*) were out-planted at both sites with experimental deployments mimicking culture methods used by the shellfish culture industry. Shellfish sampling occurred every four weeks from August 15, 2011 through November 7, 2011, and from March 5, 2012 through December 10, 2012, with one sampling point in January 2012. Following collection, shellfish samples were rinsed thoroughly in the laboratory to determine if NIS (especially *C. maenas*) were present. During each sampling event at both of the field sites, we confirmed the presence of adult (via traps), juvenile (via beach walks), and larval (via plankton hauls) Green Crabs to determine if the species was available for entrainment with shellfish.

The second study examined commercially-obtained shellfish from the west coast of Vancouver Island (supplied directly from harvesters or from processors) for the presence of NIS. Shellfish samples were brought back to the laboratory, rinsed (as in the field study), and examined for the presence of NIS (especially *C. maenas*). This observational study was conducted from August 2012 through December 2012.

A literature survey was also conducted and summarized potential mitigation measures that have been experimentally tested to reduce the potential for NIS movement on shellfish aquaculture products.

Results

Adult, juvenile, and larval *C. maenas* were found at both sites throughout the experimental period, confirming the potential for the species to be entrained in this vector.

Juvenile and megalopal stages of *C. maenas* were found on all three species of shellfish sampled (Table 1). Megalopae were found in a mussel control (*i.e.* mussels collected from a harvester that were not part of the experimental research) from June 2012 and a mussel sample collected from Sechart in September 2011. At Refuge Island, juvenile Green Crabs were found on all of the shellfish species used in this study. One juvenile was found in an August 2012 mussel sample, while two were found in suspended oyster samples (one in August 2011, the other in August 2012). Juvenile Green Crabs were also found in clam samples at Refuge Island, two in October 2012 and one in November 2012. The juveniles found on the shellfish were small, ranging in size from 1.5 to 13 mm carapace width.

Non-indigenous tunicates and bryozoans also were found during the field study, but only associated with the mussel and oyster samples, not intertidal clam samples. This is likely due to

the fact that clams were cleaned prior to deployment and to the very short storage period (days) in the intertidal zone where tunicates and bryozoans are uncommon. Species encountered included violet tunicates (*Botrylloides violaceus*), golden-star tunicates (*Botryllus schlosseri*), and the non-indigenous bryozoans *Schizoporella japonica* and *Cryptosula pallasiana*.

*Table 1. The number, life stage, and size of Carcinus maenas found on the experimental shellfish samples: * the size classes (i.e. J1 and J2) are based on the relative size frequency distribution in Silva et al. (2006) with J1–J2 being recruits and J3–J6 being early juveniles.*

Sieve fraction (mm)	Collection date (dd-mmm-yy)	Sample	Site	No. found	Life stage	CW (mm)	Comment
>0.5 – 7.5	16-Sep-11	Mussels	Sechart	1	megalop	NA	-
>0.5 – 7.5	11-Jun-12	Mussels	Control	1	megalop	NA	Not out-planted
>0.5 – 7.5	19-Aug-11	Oysters	Refuge	1	juvenile	2.5	J2*
>0.5 – 7.5	22-Aug-12	Oysters	Refuge	1	juvenile	1.5	J1
>7.5	22-Aug-12	Mussels	Refuge	1	juvenile	13	-
>7.5	18-Oct-12	Clams	Refuge	2	juvenile	7.6, 6.5	Found alive in two independent samples: J5/J6
>7.5	16-Nov-12	Clams	Refuge	1	juvenile	5.1	Alive: J4

Green Crabs were not found on any of the shellfish samples that were collected from the shellfish growers or processing plants, although various tunicate and bryozoan NIS were, including the tunicates *B. violaceus*, *B. schlosseri*, and *Didemnum vexillum*, as well as the non-indigenous bryozoan *S. japonica*. The results of both the field-based experimental study and the observational study confirm that shellfish can be a vector for NIS (including *C. maenas*).

Sources of Uncertainty

- Although our shellfish rinsing method removed much of the macrofauna, its efficacy is unknown and has not been tested. It is not known if 100% of the Green Crabs present on the samples were removed, as we had no initial measure. If the number of Green Crabs transported was under-estimated it would confound extrapolations and could under-estimate the invasion potential.
- The samples in the experimental study were not rinsed prior to transport, therefore the number of Green Crabs documented may have been higher than if industry practices and licence conditions were followed.
- Experimental sites may not be representative of *C. maenas* populations along the west coast of Vancouver Island. We chose areas that had relatively high-density populations of Green Crabs so that we could be sure that the species was present and available for entrainment. Green Crab populations are variable spatially and temporally, thereby confounding the representativeness (and hence entrainment potential) of the study.
- There are some differences between how farmers grow shellfish and how the shellfish were handled in our study, which also could influence entrainment potential. Specifically,

most shellfish are out-planted at an early stage and left largely undisturbed for a couple of years. Thus, the actual entrainment potential is a function of exposure over at least two Green Crab reproductive periods prior to harvest. In contrast, our experimental design only allowed out-planting of shellfish material that would allow entrainment over a single growing season and, in some cases, our out-plantings did not coincide with the peak Green Crab reproductive period. Thus, entrainment potential as measured by our investigation could have been underestimated relative to that of commercial practices.

- Potential uncertainties in the processor/grower observational study included: rinsing issues, lack of knowledge surrounding Green Crab populations, potential changes in standard practices, short duration (six months), and small sample sizes. According to DFO licence conditions, the samples from the observational study should have been rinsed prior to transport, but this was not confirmed and the method of rinsing was unknown. The distribution and population sizes of Green Crabs near shellfish culturing sites where the shellfish were sampled were also unknown. Although we have information about the general distribution of Green Crabs along the west coast of Vancouver Island (Gillespie et al. 2007), there is little fine-scale information on distribution or population abundance. Growers and harvesters may have also changed their operating procedures, including the treatment of shellfish before transport, in an effort to comply with licence conditions, or to demonstrate that their industry does not pose a risk of moving potential NIS. Lastly, relative to the experimental study, the sample sizes in this study were small and may not accurately reflect the potential of entrainment of mobile NIS.
- We only examined three shellfish species and two forms of culture – suspended in trays/bags and intertidal (beach) culture. Other species and other types of shellfish culture were not considered experimentally and thus their probability for entraining NIS is unknown.

CONCLUSIONS AND ADVICE

1) The transfer of shellfish (clams, oysters, and mussels) from NIS-infested areas is a vector for both mobile (*e.g.* Green Crabs) and sessile (*e.g.* tunicates/bryozoans) NIS. This is true regardless of the harvest type or intended end-use (*e.g.* commercial culture, wild harvest, or monitoring programs).
    a. Green Crabs were entrained on all three shellfish species examined.
    b. Three tunicate and two bryozoan NIS were also entrained in most of the cultured shellfish species examined.
    c. Additional species entrained included native fish, non-cultured bivalves, crabs, shrimp, macrophytes (seaweeds, seagrasses, and algae), and snails (among many others).
2) The potential propagule pressure due to the shellfish movement vector may be sufficient to overcome invasion barriers, based on historical industry production.
3) The only mechanism to ensure NIS are not inadvertently moved from infested to non-infested waters via this vector is to restrict all movement of shellfish (*i.e.* strict quarantine).
4) Based on a literature review, no mitigation measures to remove or destroy NIS on cultured shellfish are 100% effective, suggesting any transfer of shellfish poses some level of invasion risk. Use of multiple mitigation methods should reduce invasion potential relative to single control points. Some methods and their efficacy are described briefly below.
    a. Mechanical removal: hand removal and power washing. Both methods are very labour intensive and ineffective for smaller species or life stages of NIS that are not

visible to the naked eye or are hidden deep within the product. Power washing can also facilitate the spread and dominance of NIS growing at culturing/harvest sites.

b. Chemical removal: use of lime, acetic acid, or brine dips. These treatments have been experimentally tested, the results with lime showing a high percentage of removal of biofouling NIS. However, none of the treatments, including lime, effectively removed 100% of the biofouling species of interest. These treatments have not been tested on mobile NIS such as Green Crabs.

c. Biological removal: use of grazers and predator species such as sea urchins. The species tested removed biofouling NIS to varying degrees, but none fully eliminated NIS growing on shellfish. As with chemical removal, this work has largely been done on sessile NIS and its effect on mobile NIS is unknown.

5) Based on the results of the experimental and processor studies, the present conditions of licence do not eliminate NIS propagule pressure. Due to the gaps identified it is probable that the intended reduction in propagule pressure is not realized.

6) A conceptual framework was developed to identify control points where management intervention, such as application of licence conditions, could lower propagule pressure and hence invasion risk (Fig. 2). A full assessment of the relative effectiveness of each control point in the framework was beyond the scope of this project.

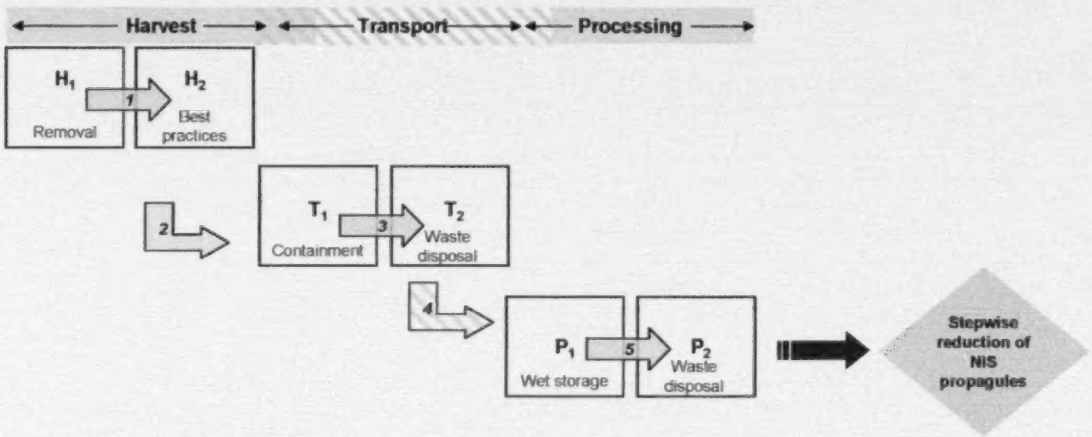
Recommendations

1) If the management objective is to fully eliminate the risk of introducing potential NIS from infested to non-infested waters on transferred shellfish, the transfer of shellfish should be halted.

2) If the management objective is to reduce the risk of introducing potential NIS from infested to non-infested waters on transferred shellfish, various mitigation measures including, but not limited to, those in the Conceptual Mitigation Framework (Fig. 2) should be invoked.

3) To facilitate further development of the conceptual framework for risk mitigation, a risk assessment needs to be undertaken to understand the relative reduction in propagule pressure at each step of the framework under various scenarios.

4) In order to determine the relative reduction in propagule pressure at each step of the conceptual framework, further experimental research is required. For instance, evaluation of the effectiveness of some of the discussed mitigations (*e.g.* power washing, chemical dips, etc.) would help to measure the relative reduction of propagule pressure of "Stage H_1: Removal" in the conceptual mitigation framework.



Category	Definition
H_1	Removal of large proportion of the growth on shellfish (e.g. biofouling, seaweeds and mobile fauna) before transport: power washing, hand scrubbing and chemical dips can be used where appropriate
H_2	Best practices: store the product away from known AIS habitats until transport (e.g. avoid mid- to high intertidal for juvenile green crab), perform a thorough visual inspection of the product before transport
T_1	Contain the shellfish, water and any growth on the shellfish during transport
T_2	Dispose of water and any growth that has sloughed off the shellfish appropriately (i.e. not near any shoreline or sewage main)
P_1	Do not wet store any shellfish from non-local waters in local waters
P_2	Dispose of, or treat any effluent and solid waste produced during shellfish processing (i.e. sea water, shellfish innards, biofouling, shells) appropriately so that live organisms cannot enter any local waters
	Overlap in responsibility between the harvester and processor exists both at the front and back end of the transport phase of shellfish; transport of product is also done by both parties

Figure 2. Conceptual framework for considering cultured shellfish movements in relation to NIS which identifies where potential mitigation measures could be applied to reduce the risk of introducing NIS to non-infested areas. AIS refers to Aquatic Invasive Species.

SOURCES OF INFORMATION

This Science Advisory Report is from the December 3-4, 2013 meeting on Evaluating transfers of harvested shellfish products, from the west to the east coast of Vancouver Island, as a potential vector for European Green Crabs *(Carcinus maenas)* and other non-native invertebrate species. Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

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Centre for Science Advice Pacific Region
Fisheries and Oceans Canada
3190 Hammond Bay Road
Nanaimo, BC V9T 6N7

Telephone: 250-756-7208
E-Mail: csap@dfo-mpo.gc.ca
Internet address: www.dfo-mpo.gc.ca/csas-sccs/

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